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Abstract

This study aimed to calibrate a productivity model for the algal species *Dunaliella salina* accounting for the impacts of light intensity and temperature. The calibration was performed by using a dedicated experimental set-up measuring short-term oxygen production rates at different light intensities. The rate of photosynthesis was shown to follow a typical Monod function of light intensity. The slope of Photosynthesis-light curves at low light intensity was also shown to be independent on temperature and the evolution of model parameters with temperature obeyed relationships consistent with previous observations in the literature. Finally, the rate of respiration was shown to follow an Arrhenius function of temperature. This good level of agreement with prior observations in the literature indirectly validates the experimental technique used for model calibration. The resulting model should therefore yield accurate productivity predictions in outdoor cultivation systems.

Keywords: Algae; *D. salina*; growth kinetics; productivity model; photosynthesis; respiration

1. Introduction

With the objective to accurately assess the economical and environmental feasibility of full-scale algal cultivation for biofuel production, a large number of studies developed mathematical models predicting algal productivity in outdoor cultivation systems [1–3]. These models can be used to improve process design or develop optimization strategies maximizing algal productivity. For instance, Slegers et al. [4] used a mathematical model predicting growth rates of *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* to optimize the design of closed photobioreactors. Similarly, Béchet et al.[5] proposed an optimization strategy based on the dynamic control of pond depth and hydraulic retention time to increase productivity while reducing water demand, using a productivity model for *Chlorella vulgaris*. Alternatively, adapting the algal species to climatic conditions could potentially boost yearly algal productivity, similarly to crop rotation used in traditional agriculture. For example, algal species having low optimal temperatures could be cultivated in colder climates or simply during winter while heat-resistant algal species could be grown in summer when pond temperature reaches higher levels. With the objective to assess the benefits of these 'algal culture rotation' strategies, it is necessary to calibrate algal productivity models for a large number of species. However, while many studies in the literature developed productivity models, these models have been calibrated on a limited number of algal species. In particular, the impact of temperature was often neglected in past studies, which limits models application to outdoor systems where temperature significantly varies [1].

Within this context, our research group has been developing mathematical models to predict algal productivity in various outdoor cultivation systems from meteorological hourly data, system design and operation. This modeling framework combined models predicting system

temperature with a biological model predicting algal productivity as a function of light and temperature. So far, the biological model has only been calibrated for a single algal species, *Chlorella vulgaris* (see Béchet et al. [6]). The objective of this study was therefore to calibrate a productivity model for another algal species, *Dunaliella salina*, this species being the third most cultivated microalgae [7]. *Chlorella vulgaris* and *Dunaliella salina* are both Chlorophyceae and share the same tolerance to high temperatures. The methodology followed in this study was therefore similar to the calibration technique followed by Béchet et al. [6], and also because the model for *C. vulgaris* accurately predicted productivities in indoor (accuracy of +/- 15% over 163 days; Béchet et al. [6]) and outdoor (accuracy of +/- 8.4% over 148 days, Béchet et al. [8]) reactors.

2. Materials and methods

2.1. Algae cultivation conditions and biomass characterization

The Chlorophyceae *Dunaliella salina* (CCAP 19/18) was cultivated in a cylindrical photobioreactor (diameter: 0.19 m; height: 0.41 m; culture volume: 10 L; gas phase volume: 1.6 L). The reactor was illuminated by two metal halide lamps (Osram Powerstar HQI-TS, 150W NDL, Neutralweiss de Luxe) providing a light intensity of 1440 μmol/m^2-s (measured when the reactor was filled with water with a QSL-2100 PAR scalar irradiance sensor, Biospherical Instruments). Temperature was maintained at 30ºC by re-circulating temperature-controlled water in a jacket around the reactor. The reactor was inoculated with a culture of *D. salina* (inoculum volume of approximately 500 mL) grown in axenic conditions (light intensity: 300 μmol/m^2-s, light-dark cycle: 12:12, temperature: 27ºC) and was then operated as a fed-batch system: 9L of solution was replaced every week with fresh f/2 medium [9] enriched in phosphorus and nitrogen to ensure that algal growth was not limited by nutrients (Total N and P concentrations in enriched medium: 0.22 g N-NO_3^-/L; 0.020 g P-

PO_3^-/L). Air enriched in CO_2 (2% CO_2) was continuously bubbled in the photobioreactor to ensure that CO_2 supply did not limit algal growth and also to control pH between 7 and 7.5. Algae used for model calibration were extracted from the photobioreactor 2-3 days after medium change during the light-limited growth phase. The biomass concentration in the solution used during model calibration was measured by dry weight [10]. Glass-fiber filters (GF/C, Whatman, diameter: 25mm, No 1822-025) were first dried for several days at 60°C before being weighed. A known volume of the algal solution was then filtered; filters were then rinsed with Ammonium formate (30 g/L) to remove salt. Filters were then dried for 24 hours at 60°C before being weighed again. Dry weight concentration was measured in duplicates.

2.2. Productivity model description

Algal productivity (P_{net}, in kg O_2/s) was expressed as the difference between the gross rate of photosynthesis (P, in kg O_2/s) and the rate of endogenous respiration (ER, in kg O_2/s) [6]:

$$P_{net} = P - ER \quad (1)$$

The gross rate of photosynthesis was expressed as a function of light intensity and temperature by using a 'type-II model' as recommended by Béchet et al. [1]. This type of models is based on the assumption that the local rate of photosynthesis of single algal cells can be expressed as a direct function of the local light intensity cells are exposed to. Béchet et al. [6] used different formulas to express local rates of photosynthesis as a function of local light intensity and showed that the three formulas most commonly used in the literature all satisfyingly fit experimental data. The authors finally selected the Monod formula, as this expression was the most commonly found in literature. The gross rate of photosynthesis was therefore expressed as [6]:

$$P_{net} = \int_V P_m(T) \frac{\sigma_X I_{loc}}{K(T)+\sigma_X I_{loc}} X \cdot dV \quad (2)$$

where P_m is the maximum specific rate of photosynthesis (kg O_2/kg-s), T is the culture temperature (°C), K is the half-saturation constant (W/kg), σ_X is the extinction coefficient (m^2/kg), I_{loc} is the local light intensity (W/m^2, as photosynthetically active radiation or PAR), X is the algal concentration (kg/m^3) and V is the culture volume (m^3). The local light intensity I_{loc} was expressed by using a Beer-Lambert law:

$$I_{loc}(l) = I_0 exp(-\sigma_X X l) \quad (3)$$

where l is the light path between the considered location and the reactor external surface (m) and I_0 is the incident light intensity (W/m^2). The evolution of P_m and K with temperature was fitted to the theoretical model of Bernard and Rémond [11] as this model was shown to satisfyingly fit the evolution of the specific growth rate of 15 algal species. P_m and K were therefore expressed as follows:

$$p = \begin{cases} \alpha \frac{(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})((T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T))} & \text{if } T_{min} \leq T \leq T_{max} \\ 0 & \text{otherwise} \end{cases} \quad (4)$$

where p represents either P_m or K, α is the maximum value of P_m or K, and T_{min}, T_{max} and T_{opt} are the minimum, maximum, and optimum temperatures for photosynthesis (°C), respectively. The rate of endogenous respiration was expressed using a first-order law:

$$ER = \lambda(T) X V \quad (5)$$

where λ is the specific respiration rate (kg O_2/kg-s). Several studies showed that the rate of respiration was an exponential function of temperature [12–14]. The parameter λ was therefore expressed as follows:

$$\lambda(T) = \lambda_0 exp(\beta T) \quad (6)$$

where λ_0 (kg O_2/kg-s^{-1}) and β (°C^{-1}) are determined experimentally.

2.3. Device used for model calibration

The device used for calibration was composed of six cylindrical glass reactors (diameter: 3.48 cm; height: 5.82 cm; volume: 55.2 mL) all equipped with an oxygen electrode (Model DO50-GS, Hach) measuring both dissolved oxygen and medium temperature. Each reactor was positioned over a LED lamp (12V PHILIPS EnduraLED 10W MR16 Dimmable 4000 K) which light intensity was independently controlled. A typical experiment consisted on measuring first oxygen production rates when algae were exposed to light (light-phase) and then respiration rates when algae were in the dark (dark phase). These measurements were performed for six different light intensities (range: 0-460 W/m^2, as PAR) and under constant temperature (see [6] for a complete description of the oxygen measurements). These experiments were then repeated for 6 different temperatures (3.73°C; 10.2°C; 19.7°C, 27.7°C, 34.7°C; 40.9°C). Temperature was maintained constant (within approximately +/- 1°C) during the entire duration of the experiment by circulating temperature-controlled air around the reactors. The light intensity reaching the external surface of each reactor was measured by actinometry (see S1 for details). The parameters P_m and K for each temperature were determined by least-square fitting using the *lsqcurvefit* Matlab function. Respiration rates during the dark periods were found to be independent on the light intensity cells where exposed to during the light phase and the parameter λ was determined from the average respiration rate in the six reactors. The parameters T_{min}, T_{max}, T_{opt}, were obtained by least-square fitting (using the *lsqcurvefit* Matlab function) and the parameters λ_0 and β were estimated by log-linear regression. Algae were found to be photosynthetically inactive after exposure to 43°C for 30 min (unpublished data). P_m was therefore considered null at 43°C when determining T_{min}, T_{max}, and T_{opt}. Based on the linear relationship between P_m and K (see section 3.1 for details), K was also assumed null at this temperature.

2.4. Measurement of extinction coefficient

The extinction coefficient σ_X was experimentally determined by measuring the light intensities entering and exiting the reactors for different algal concentrations (see S2 for details). Similarly to the formula proposed by [15], the extinction coefficient was expressed as follows:

$$\sigma_X = AX^B \quad (7)$$

where A and B are empirical coefficients (A = 79.1; B = -0.37, see S2 for details). The dependence of the extinction coefficient on algal concentration was mostly due to light scattering by algal cells. Scattered photons indeed exited the reactors through the lateral side of the reactors. This effect was reinforced by the fact that LEDs lamps did not emit light in a vertical direction but in a cone of an angle 30°, which increases the fraction of light lost through the reactors lateral sides. When the algal concentration increased, most of photons were absorbed by algal cells and the fraction of light exiting the reactors through the lateral sides decreased. This explains why the extinction coefficient is less sensitive to X for high algal concentrations (Equation 7; see S2 for further detail). Calibration experiments were therefore performed at relatively high algal concentrations to ensure that most of incoming light was absorbed by algae.

2.5. Application to the calibration device

Based on Equations 1-7, the algal productivity in each reactor used for model calibration was expressed as follows:

$$P_{net}(T, I_0) = \frac{P_m(T)S}{\sigma_X} ln\left(\frac{K(T)+\sigma_X I_0}{K(T)+\sigma_X I_0 \cdot exp(-\sigma_X XL)}\right) - \lambda(T)XSL \quad (8)$$

where T is the culture temperature (°C), I_0 is the incident light intensity at the reactor bottom (W m^{-2}), P_m, K and λ are the temperature-dependent model parameters (see Equations 4 and

6), σ_X is the extinction coefficient (see Equation 7), X is the algal concentration (kg m^{-3}), and L and S are the reactor height (m) and section surface area (m^2), respectively.

2.6. Monte-Carlo simulations

Monte-Carlo simulations were performed to quantify the impact of experimental error on the fitted values of model parameters P_m, K and λ. Namely, four key measurements were found to have a significant impact on model parameters:

- The extinction coefficient σ_X: coefficients A and B in Equation 7 were found to vary in the ranges 74.50-82.78 and -0.20--0.48, respectively (see S2 for details);
- The dissolved oxygen concentration: oxygen probes were found to be accurate at +/- 4.7% (see S3 for details);
- The incident light intensity I_0: Measurements by actinometry were assumed to be accurate at +/-10% based on the study of Hatchard and Parker [16] (See S1 for details).
- The algal concentration X: an accuracy of +/-7% on dry weight measurements was assumed by analogy with the study of Béchet et al. [6].

In practice, the uncertainties on the parameters P_m, K and λ were obtained from the following Monte-Carlo approach. Assuming that errors were normally distributed, a large artificial data set was generated by adding a normally distributed error to the measurements (algal concentration X, light intensity I_0 and oxygen concentration) and the extinction coefficient (σ_X). A total of 2000 artificial data sets were thus generated and the parameters P_m, K and λ were determined with a minimization algorithm for each data set. This approach yielded a normal distribution for P_m, K and λ, which allowed determining confidence intervals for each of these parameters. These resulting confidence intervals were then used to determine levels

of uncertainty on the parameters T_{min}, T_{max}, T_{opt}, λ_0 and β through another set of Monte-Carlo simulations (see [6] for further details on Monte-Carlo simulations).

2.7. Conversion coefficients from oxygen to biomass productivity

The productivity model developed in this study predicts algal productivity in terms of oxygen (see Equations 1-6). For engineering purposes, it is however necessary to express productivities in terms of biomass. The conversion from oxygen to biomass productivities was performed by following the approach of Béchet et al. [6]. This conversion was based on the assumption of a photosynthetic quotient of 1 mole of CO_2 consumed for the production of 1 mole of O_2 during the light reactions of photosynthesis, which was supported by the experimental measurement of the photosynthetic quotient of a close algal species (*Dunaliella tertiolecta*) by Wegmann and Metzner [17]. This photosynthetic quotient of 1 does not include respiratory mechanisms and only reflects photosynthesis. From the knowledge of algal composition and by considering that nitrate was used as a nitrogen source, the following conversion coefficients were obtained (see S4 for details):

- P_m' [kg/kg-s] = 0.75 (± 0.10) P_m [kg O_2/kg-s]
- λ' [kg/kg-s] = 0.75 (± 0.10) λ [kg O_2/kg-s] at daytime
- λ' [kg/kg-s] = 0.9375 (± 0.10) λ [kg O_2/kg-s] at nighttime

3. Results and discussion

3.1. Rate of photosynthesis

Figure 1 shows that the Type-II model coupling a Monod formula with the modified Beer-Lambert law was able to describe the evolution of the rate of photosynthesis with light intensity. These PI-curves do not exhibit the typical decrease at high light intensities due to

223 photo-inhibition observed for *D. salina* in dilute cultures through chlorophyll fluorescence
224 measurements by Combe et al. [18]. This is explained by the high algal concentration that
225 ensured that only a small fraction of cells were photo-inhibited, so that the impact of photo-
226 inhibition was minimal, as suggested by Bernard [19]. Experimental errors caused relatively
227 high uncertainty on fitted values of P_m and K as shown in Table 1 and especially at the
228 temperature of 40.9°C as the gross productivity was only measured at two light intensities (the
229 oxygen net productivity was negative at low light intensities due to high respiration rates at
230 this temperature, and oxygen concentration remained null during all experiment). Because of
231 these experimental uncertainties, it was difficult to accurately identify P_m and K separately. In
232 other words, various combinations of P_m and K could yield equally satisfying fits in Figure 1.
233 In spite of these levels of inaccuracy, Figure 2 shows that P_m and K were correlated (R^2 =
234 0.87558), which was previously observed by Béchet et al. [6]. The ratio P_m/K indeed
235 represents the maximum 'yield' of photosynthesis (in kg O_2/W-s), i.e. the amount of oxygen
236 produced through photosynthesis per unit light energy captured by cells. For low light
237 intensities, this maximum yield is theoretically independent of temperature [20], which
238 explains the linearity observed in Figure 2.
239
240 Figure 3 shows that experimental values of P_m followed a typical response to temperature
241 characterized by a slow increase from cold to optimal temperatures before a fast drop for
242 higher temperatures. The model of Bernard and Rémond [11] especially developed for this
243 type of temperature response thus provides a good fit to experimental data (Figure 3).
244 Interestingly, the model of Bernard and Rémond successfully described the evolution of K,
245 with similar values for T_{min}, T_{max}, and T_{opt} as shown in Table 2. This similarity is explained by
246 the linearity between P_m and K shown in Figure 2. The values of T_{min}, T_{opt} and T_{max} are within
247 the range of values obtained by Bernard and Rémond [11] for 15 other algal species. The

value of the maximum temperature T_{max} is in the upper range of reported values for other algal species, which may be explained by two reasons. Firstly, *D. salina* is known to resist to high temperatures as this species is naturally found in shallow water bodies in which temperature can reach relatively high values [21,22]. In addition, this model calibration is based on short-term measurements of photosynthesis (approximately 30 min) while Bernard and Rémond [11] fitted their model on growth rate measurements obtained over several days of cultivation. Even if Bernard and Rémond [11] did not calibrate their model on *D. salina* data, this difference in time scales may explain the relatively high T_{max} value (43°C) as short-term and long-term algal responses are not controlled by the same biological processes. Oxygen production indeed reflects the rate of initial non-enzymatic steps of photosynthesis (usually referred to as "light reactions"), while carbon fixation through Calvin cycle and more generally growth involve enzymatic processes that are more impacted by temperatures. For example, Béchet et al. [6] showed that *Chlorella vulgaris* was unable to sustain growth at a constant temperature of 35°C for more than 1-2 hours while the oxygen productivity peaked at 38°C. The uncertainty on P_m and K showed in Table 1 caused levels of uncertainty on T_{max}, T_{min} and T_{opt} similar to the confidence intervals presented by Bernard and Rémond [11], even if methods for uncertainty estimations were based on different approaches (Table 2).

3.2. Respiration rate

Figure 4 shows that the specific respiration rate increased exponentially with temperature over the range of temperatures tested. Similar observations were reported for a large number of algal species as reviewed by Robarts and Zohary [23]. Based on the values reported in Table 1, the coefficients λ_0 and β (with corresponding confidence intervals at 95%) were $6.45\ 10^{-7}$ ($+/- 0.34\ 10^{-7}$) s^{-1} and 0.0715 (+/- 0.0002) $°C^{-1}$, respectively.

4. Conclusions

The results obtained during the model calibration performed on *D. salina* are consistent with prior observations in the literature, namely:

- The rate of gross oxygen productivity followed a typical Monod-like response to light intensity;
- The maximum specific rate of oxygen production was linearly correlated to the half-saturation constant of the Monod model, indicating that oxygen production efficiency is as expected independent of temperature at low light intensities;
- The evolutions of the maximum specific rate of photosynthesis and half-saturation constant with temperature satisfyingly fitted Bernard and Rémond's model.
- Respiration rates were shown to increase exponentially with temperature, which is consistent with prior observations in the literature.
- These results also confirm that *Dunaliella salina* can grow in a relatively wide temperature range and resist to relatively high temperatures.

These results indicate that the experimental technique used for model calibration is valid and that the productivity model should yield accurate predictions in outdoor cultivation systems.

Acknowledgments:

The authors are grateful for the support of the ANR-13-BIME-004 Purple Sun and the Inria Project Lab *Algae in silico*. Margaux Caïa (Inria BIOCORE/LOV) is acknowledged for early work on the device used for model calibration.

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Table 1: Model parameters values at different temperatures (values in parenthesis indicate confidence level at 95% estimated through Monte-Carlo simulations). Values of P_m and λ in kg/kg-s can be obtained by using the conversion coefficients provided in Section 2.6. Values of K can be obtained in μmol/kg-s by using a conversion factor of 4.79 μmol/W-s (based on the spectral distribution of device lamps shown in S1).

Temperature (°C)	3.7	10.2	19.7	27.7	34.7	40.9
P_m (10^{-4} kg O_2/kg-s)	0.165 (0.014)	0.571 (0.096)	1.19 (0.25)	2.08 (0.43)	1.77 (0.33)	1.35 (0.88)
K (10^4 W/kg)	0.479 (0.095)	2.46 (0.64)	2.79 (0.87)	4.77 (1.34)	3.34 (0.89)	3.28 (3.31)
λ (10^{-6} kg O_2/kg-s)	0.88 (0.05)	1.22 (0.06)	2.55 (0.13)	5.45 (0.28)	7.53 (0.39)	11.5 (0.6)

371 Table 2: Bernard and Rémond's model parameters for P_m and K (values in parenthesis indicate
372 confidence interval at 95% estimated through Monte-Carlo simulations) - Symbols are
373 defined in Equation 4. Values of α for K can be obtained in μmol/kg-s by using a conversion
374 factor of 4.79 μmol/W-s

Parameter (unit)	T_{min} (^oC)	T_{opt} (^oC)	T_{max} (^oC)	α
P_m (kg O_2/kg-s)	-7.8 (8.4)	34.0 (3.9)	43.0 (0.1)	2.08 10^{-4} kg O_2/kg-s
K (W/kg)	-15.4 (17.3)	33.7 (8.0)	43.0 (0.4)	4.77 10^4 W/kg

375

Figures

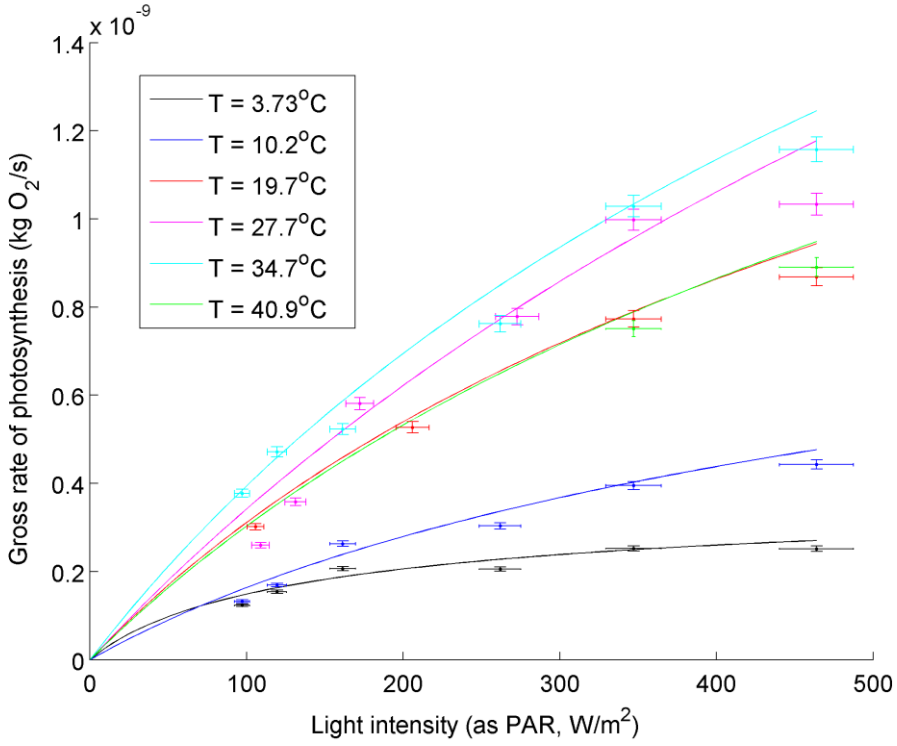
Figure 1: Gross rate of photosynthesis vs. incident light intensity at different temperatures (dots: experimental data; plain lines: theoretical fitting) - Error bars represent standard deviation of error caused by experimental error. Light intensities in W/m^2 can be converted into $\mu mol/m^2$-s by using a conversion factor of 4.79 μmol/W-s.

Figure 2: Values of the maximum specific growth rate P_m vs. the half-constant K (dots: experimental data; plain line: linear regression) - Error bars indicate the standard deviation estimated through Monte-Carlo simulations.

Figure 3: Evolution of the maximum specific oxygen productivity and half-saturation constant with temperature (dots: experimental data; plain line: fitting with Bernard and Rémond's model) - Error bars represent the standard deviation estimated through Monte-Carlo simulations. Values of K can be obtained in μmol/kg-s by using a conversion factor of 4.79 μmol/W-s.

Figure 4: Evolution of the respiration specific rate with temperature (dots: experimental data; plain line: fitting to an exponential function as described by Equation 6) - Error bars represent standard deviation estimated through Monte-Carlo simulations.

396 Figure 1



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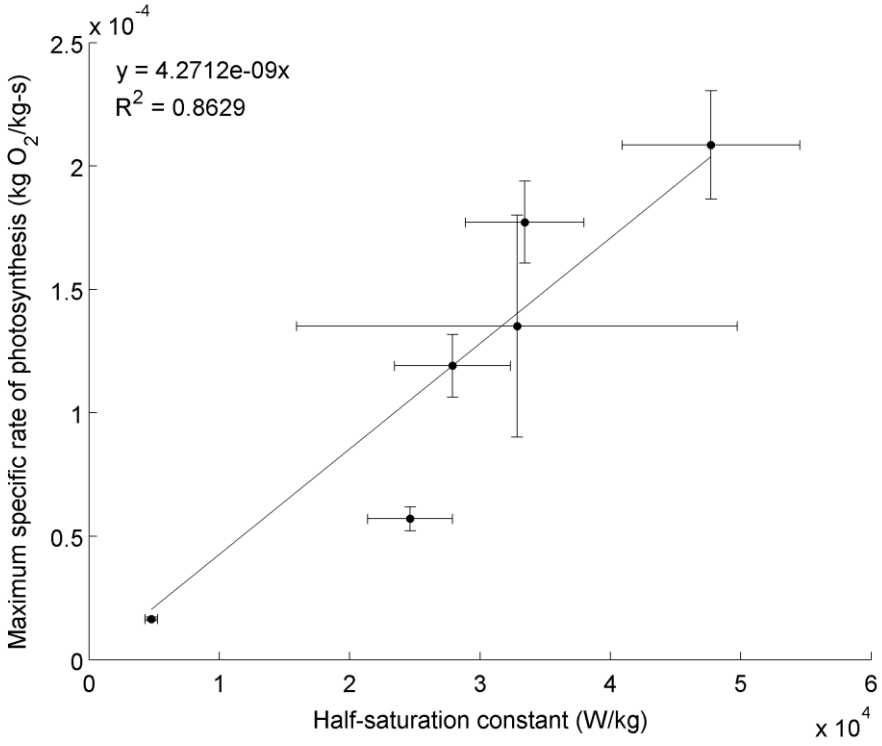
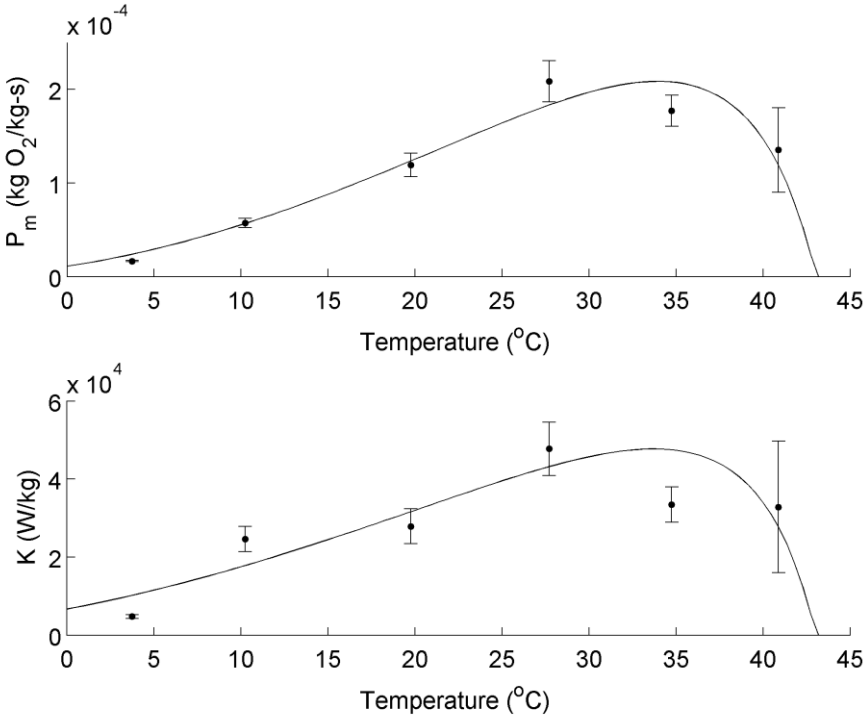
398 Figure 2



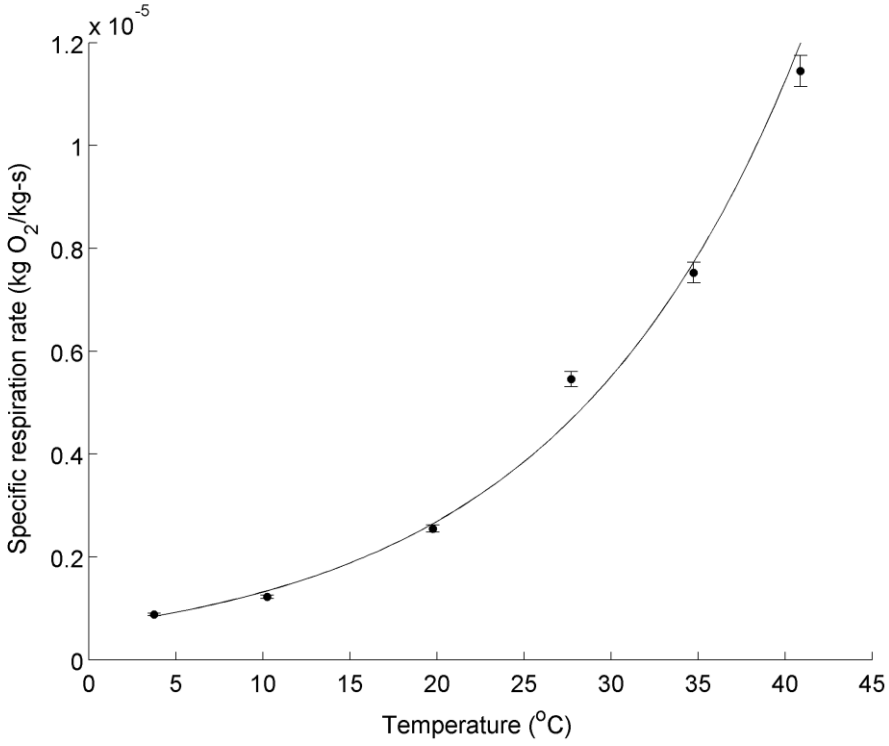
399

400 Figure 3



401

402 Figure 4



403